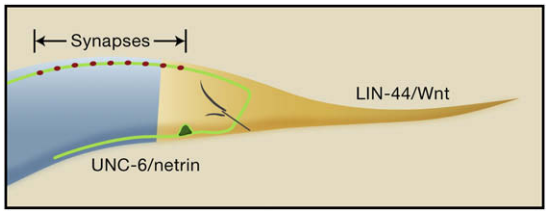
Neurobiology Select

Cell

Migration of neurons is crucial for brain development. Equally essential to this developmental process is the specific transport of proteins within the neuron for axonal and dendritic specification. This Neurobiology Select highlights new findings that shed light on the mechanisms regulating both the movement of proteins within neurons and the movement of neuronal cells themselves within the developing brain.

An UNConventional Role for Netrin



Wnt (orange) and Netrin (blue) gradients control subcellular localization of synaptic components (red) in the DA9 neuron (green) of the worm by inhibiting synapse formation. Image courtesy of V.Y. Poon and K. Shen.

The establishment of neuronal polarity requires the correct distribution of proteins that determine the specialized identities and functions of axons and dendrites. In a new study, Poon et al. (2008) show that extracellular cues that mediate axon guidance may also play a role in directing this process. In the DA9 motor neuron of the worm *Caenorhabditis elegans*, presynaptic proteins such as the synaptic vesicle-associated protein RAB-3 are found in the dorsal axon and excluded from the dendrites. However, in the absence of the conserved axon guidance molecule Netrin (UNC-6) or its receptor (UNC-5), these presynaptic proteins are mislocalized to the dendrites. By manipulating the activity of UNC-5 at different times during development, the authors uncovered evidence for a new Netrin receptor function distinct from that of axon guidance during development. They show that inactivation of UNC-5 in the fully developed DA9 neuron induced irreversible mislocalization of presynaptic proteins. To determine whether signaling to UNC-5 by UNC-6 directly mediates the localization of presynaptic proteins, Poon et al. examined green fluorescent protein-tagged RAB-3 (GFP-RAB-3) in worms engineered to express a posterior to anterior gradient of UNC-6. In these worms, the authors observed a displacement of GFP-RAB-3 towards the anterior of the DA9 neuron, away from the posterior portion of the DA9 dorsal axon that is exposed to the highest level of UNC-6. This GFP-RAB-3 displacement requires UNC-5, strongly suggesting that UNC-5 activation in the DA9 neuron by extracellular UNC-6 does indeed direct presynaptic protein localization. Interestingly, the authors found further evidence that Wnt (LIN-44), another secreted signaling molecule, could play a role similar to UNC-6 in directing presynaptic protein localization. How these two distinct signaling pathways may be coordinated in the spatial regulation of neuronal proteins remains an exciting question for future exploration.

V.Y. Poon et al. (2008). Nature ***425****, 669–674.*

A CALMing Influence on Neurite Development

Another mechanism by which proteins can be differentially distributed between axons and dendrites is through endocytosis. Bushlin et al. (2008) now report findings that suggest roles for the clathrin assembly proteins AP180 and CALM (clathrin assembly lymphoid myeloid protein) in the development of axons and dendrites in the embryonic rat hippocampus. Because AP180 and CALM are known to be involved in clathrin-mediated endocytosis and are highly expressed in the young hippocampal neurons of rats, Bushlin and colleagues examined the effects of depleting either gene by small-interfering RNAs in cultured rat hippocampal neurons. These cultured neurons possess 5–6 projections from the cell body (neurites) consisting of one axon and multiple dendrites, as confirmed by the expression of dendrite- and axon-specific markers. AP180 depletion resulted in specific loss of the axon in most neurons, whereas neurons lacking CALM tended to maintain the axon but lose their dendrites. Immunogold electron microscopy of hippocampal neurons showed that unlike AP180, which clusters at synaptic vesicles or is dispersed throughout the cytoplasm, CALM is concentrated at endosomes, supporting the notion of different functional roles for each protein. Consistent with this, secretory transport of a fluorescently labeled viral coat protein in neurons lacking AP180 or CALM revealed a specific requirement for CALM in transport of the protein to the cell surface. Despite these distinctions that suggest differing functions for these two proteins, AP180- or CALM-depleted neurons both showed similar aberrant distribution of VAMP2, an axonal synaptic protein that is normally selectively removed from dendrites by endocytosis. This implies that both proteins likely still share a common function in mediating protein distribution. Future work will be required to build on these interesting findings to ascertain the precise contributions to neurite development by AP180 and CALM.

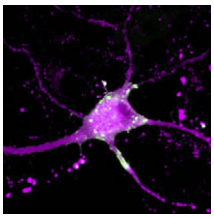
I. Bushlin et al. (2008). J. Neurosci. ***28****, 10257–10271.*

A Missing Link in Axonal Transport

Synaptic proteins are synthesized in the neuronal cell body and transported to the synapse in vesicles. The motor proteins, kinesin superfamily protein 1A (KIF1A) and KIF1Bβ, mediate the movement of these precursors to mature synaptic vesicles along the axon to the synapse. Niwa et al. (2008) now report new insights into the mechanisms of how this protein trafficking process is regulated using the vesicle cargo protein Rab3 as an example. The small GTPase protein Rab3 is found on synaptic vesicles and is required for exocytosis and release of neurotransmitter at the synapse. Although its association with the KIF motor proteins has been suggested, the vesicle-binding domains of both KIF1A and KIF1Bβ were thought to be too weak to mediate efficient transport without an adaptor protein. Using a yeast two-hybrid screen, Niwa and colleagues identified the protein DENN/MADD (differentially expressed in normal and neoplastic cells/MAP kinase-activating death domain) as the putative adaptor between Rab3 and the KIF motor proteins. DENN/MADD physically interacts with KIF1A and KIF1Bβ but not with a related KIF motor protein. Crucially, depletion of DENN/MADD from

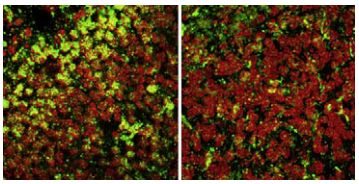
cultured hippocampal neurons reduced the amount of Rab3 transport to distal axons and mostly abolished the interaction between Rab3 and KIF1Bβ, as assayed by coimmunoprecipitation, thus confirming DENN/MADD as the link between motor and cargo. Using mutant forms of Rab3 that mimic either the GTP- or GDP-bound state of the protein, the authors found that DENN/MADD exhibited preferential binding to Rab3-GTP. Indeed, mutant Rab3 protein mimicking the GTP-bound state was more efficiently transported than mutant Rab3 mimicking the GDP-bound state. Together, these data suggest a mechanism of cargo selection by motors via adaptor proteins. The authors propose that axonal transport can be regulated via the nucleotide-bound state of the cargo.

S. Niwa et al. (2008). Nat. Cell. Biol. Published online October 12, 2008. 10.1038/ncb1785.



GTP-Rab3 (purple) is more efficiently transported than GDP-Rab3 (green) by the KIF1A and KIF1Bβ motors in a hippocampal neuron. Image courtesy of S. Niwa, Y. Tanaka, and N. Hirokawa.

Migrating Neurons Reelin Notch

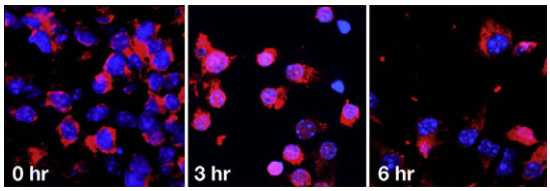


Active Notch (green) is reduced in the nuclei (red) of neurons in the developing cortex of the *reeler* mouse (right) relative to the control (left). Image courtesy of K. Hashimoto-Torii.

During brain development, signaling mediated by the secreted glycoprotein Reelin controls the radial migration of neurons, a process essential for the proper formation of neuronal layers in the neocortex. A new report by Hashimoto-Torii et al. (2008) now describes a requirement for Notch signaling in Reelin-directed cell migration within the mouse embryonic neocortex. Notch signaling is known to direct developmental events in the neocortex, but in regions distinct from those regulated by Reelin. However, Hashimoto-Torii and colleagues observed that neurons in mice lacking Reelin (*reeler* mice) also exhibit decreased Notch signaling—the level of the active Notch receptor, Notch1, was markedly lower and Notch-activated reporter gene expression was barely detectable. The authors further found that reduction of Notch signaling in mice through the deletion of two Notch receptor genes disrupted neuronal migration and resulted in an abnormal neocortex structure similar to that observed in *reeler* mice. Remarkably, overexpression of the active form of Notch1 in *reeler* mice rescued the morphological defects of *reeler* neurons that disrupted neuronal migration, suggesting that Notch activation may be the predominant function of Reelin in this process. To elucidate the molecular basis of the link between these two signaling pathways, the authors examined the interaction between the downstream effectors of Reelin, Disabled 1 (Dab1) and Notch1. Coimmunoprecipitation experiments confirmed a physical interaction between Dab1 and the active form of Notch1; in vitro experiments in cultured cells suggested that activated Dab1 protected active Notch1 from proteasomal degradation. Indeed, analysis of wild-type and *reeler* mouse brain slices showed an increase in polyubiquitinated Notch1 in the absence of Reelin. Although it remains to be determined how Reelin-mediated Notch signaling directs neuronal migration, Hashimoto-Torii et al. have made an exciting first step in tying together these two signaling pathways in this process.

*K. Hashimoto-Torii et al. (2008). Neuron **61**, 273–284.*

Sirt1 Takes a Nuclear Jaunt to Direct Neuronal Differentiation



Sirt1 (red) in cultured neural precursor cells 0 hr, 3 hr, or 6 hr after transfer into differentiation conditions. Nucleus, blue. Image courtesy of Y. Horio.

In the developing mammalian brain, neural precursor cells (NPCs) sequentially differentiate into neurons, astrocytes, and oligodendrocytes. The transcriptional inhibitor Hes1, induced by Notch signaling, mediates maintenance of NPCs and blocks this differentiation process. Hisahara et al. (2008) now suggest that the transient translocation of the histone deacetylase Sirt1 into the NPC nucleus circumvents this inhibition to modulate neuronal differentiation in mice. *Sirt1* is strongly expressed in NPCs of the mouse embryonic brain. Although it is known to be located in the nucleus of cultured muscle stem cells, the authors found that Sirt1 is predominantly located in the cytoplasm of NPCs. Using both immunostaining and live imaging of green fluorescent protein-tagged Sirt1, Hisahara and colleagues made the startling observation that 10 min after the introduction of cultured NPCs to differentiation-inducing conditions, Sirt1 began to translocate to the nucleus. Three hours after full nuclear localization, Sirt1 disperses back into the cytoplasm. Either chemical inhibition of Sirt1 activity or the expression of a dominant-negative *Sirt1* allele in cultured clusters of NPCs (neurospheres) under differentiating conditions resulted in decreased differentiation of NPCs into neurons. Similar defects in differentiation were observed in neurospheres expressing a mutant Sirt1 protein constitutively sequestered in the cytoplasm, indicating that the nuclear translocation of Sirt1 mediates its role in neuronal differentiation. The authors found that the nuclear receptor corepressor (N-CoR), a known Sirt1-interacting protein in adipocytes, also physically interacted with Sirt1 in embryonic brain cells. Overexpression of both *N-CoR* and *Sirt1* in differentiating neurospheres dramatically elevated the number of neurons formed. Hisahara et al. further determined that Sirt1 and N-CoR bound to the promoter of the NPC differentiation inhibitor Hes1 and seem to cooperate to repress its expression. These data thereby establish a molecular model for future studies of the regulation of neuronal differentiation.

*Hisahara et al. (2008). Proc. Natl. Acad. Sci. USA **105**, 15599–15604.*

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